

Supplementary Materials for
**Quantitative modulation of a spatial enhancer through the biophysical
properties of a transcription factor binding site**

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Sci. Adv. **12**, eadz5902 (2026)
DOI: 10.1126/sciadv.adz5902

The PDF file includes:

Figs. S1 and S2
Legends for tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S3

A

construct	sequence	affinity
I-WT	ATAAATTAAT	25.5
I-14	GGAAATTATA	13.8
I-18	GGTCATTACC	18.4
I-20	ATAAATTATC	20.0
I-24	GGTAATTGTT	23.8
I-33	CATAATTGTC	32.8
I-40	GGTAATTGGA	40.4
I-51/F51	GCCAATTAGC	51.4
I-66	GCCAATTATA	65.5
I-75	GGTAATTAGA	74.8
I-87	GGTAATTATA	87.0

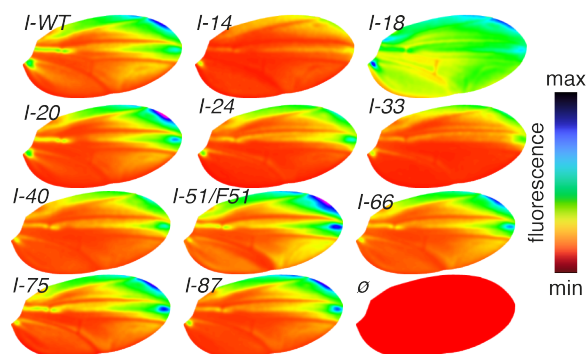
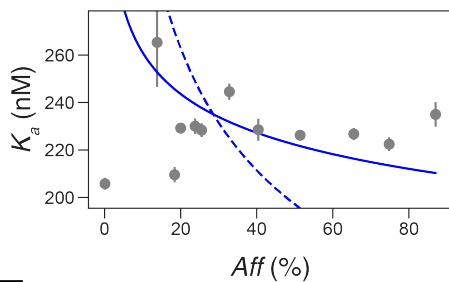
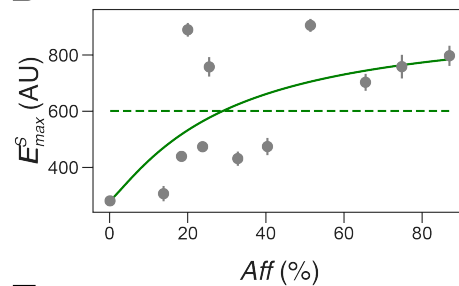
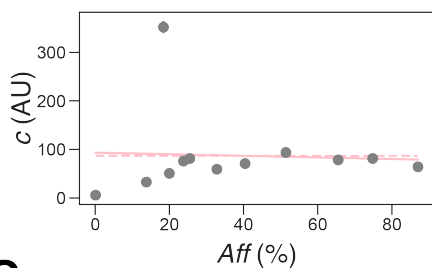
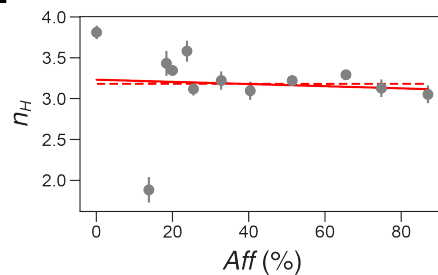
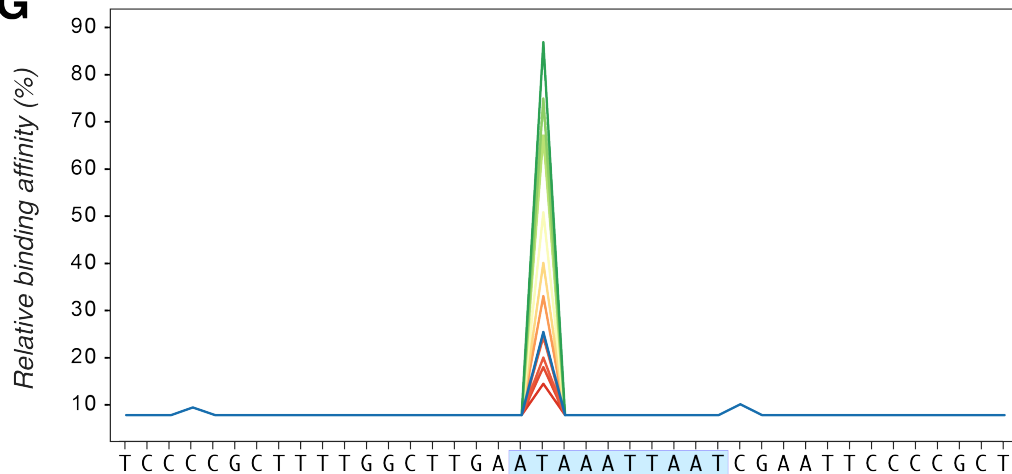
B**C****D****E****F****G**

Fig. S1.

Reporter-construct design and phenotypes for original series. (A) Full set of *Dll-c* variants showing their sequence and binding preference according to SELEX-seq. Construct names indicate that they are from the initial series (I) and their relative binding affinity is provided as a percentage. I-WT is the endogenous *Dll-c* sequence. (B) Average wing phenotypes showing the average reporter signal from tens of wings expressing each construct. (C–F) (As in Fig. 3E–H) Hill parameters for each *Dll-c* variant. Error bars show the standard deviation of parameter values determined from 100 iterations of generating average phenotypes from random sample ($n = 30$) of wings from each variant. Dotted lines indicate expected relationships between relative affinity and each parameter and solid lines indicate best fitted relationship between relative affinity and each parameter. (G) Predicted Dll binding sites of construct. Peaks indicate the 5'-most position where Dll is predicted to contact the DNA at each site. Colors indicate the relative binding affinity of each site, with green representing the highest and red the lowest affinity. The blue line refers to the predicted binding sites for the wild-type sequence. The blue box indicates the position of the *Dll-c* binding site. The blue peaks 5' and 3' of *Dll-c* show alternate binding sites which may be responsible for the weak relationship between *Aff* and expression.

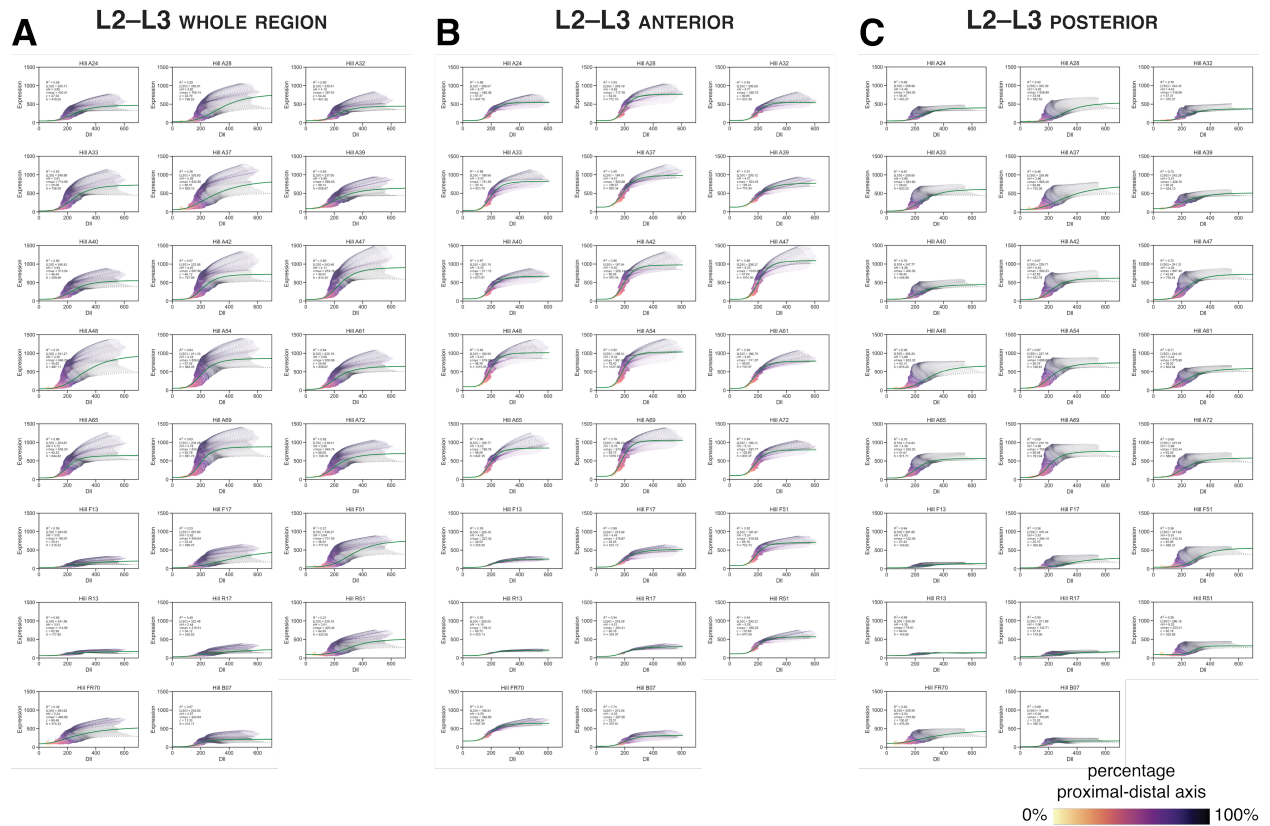


Fig. S2.

Plots of Dll concentration against average reporter signal for each enhancer variant to which a Hill equation is fitted (green line). The parameters from this fit provide the parameter value for Figs. 3, 4 and 6. (A) Plots using the entire L2-L3 region. (B) Plots using the anterior half of the L2-L3 region. (C) Plots using the posterior half of the L2-L3 region. The color scale shows the relative distance of each point along proximal-distal axis within each region.

Table S1.

SELEX-seq data showing 8-mer sequences and relative affinities of all sequences normalized to the highest affinity sequence.

Table S2.

Binding affinity and importance scores for Arg128A, Gln173A, Asn174A, Lys169A and Arg125A, with A indicating the chain, for different binding site variants using DeepPBS.

Table S3.

Sequences, affinities and number of wings imaged for each enhancer variant, Dll-reporter lines and controls.